The Male Reproductive Organs of *Nezara viridula* (L.) with a Preliminary Account of Their Development. (Heteroptera; Pentatomidae)

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Abstract

The male reproductive organs of *Nezara viridula* are described. Special attention is paid to the structure of the bulbus ejaculatorius, which is very complex in the Pentatomidae. Developmental studies show that all epithelia of the bulbus are of ectodermal origin, being produced by the elaboration of the wall of the ductus ejaculatorius.

Introduction and Acknowledgments

The male reproductive organs of the Pentatomidae are, in general, more complex than those of other families of the Heteroptera. This complexity is apparent in the structure of the bulbus ejaculatorius and in the presence of often elaborate ectodermal accessory glands (ectadenes) in addition to the mesodermal accessory glands (mesadenes).

The green vegetable bug *Nezara viridula* was chosen for study because of its abundance in Auckland. The insects were fixed in Bouin either before or after dissection, and preserved in 80% alcohol. The investigation was carried out in the Department of Zoology and Applied Entomology in the Imperial College of Science, London.

The author wishes to acknowledge the helpful advice of Professor O. W. Richards and Mr. R. G. Davies, of Imperial College. These studies formed a part of a larger investigation on the male and female reproductive organs of the Heteroptera. It is intended to publish a condensation of this investigation elsewhere.

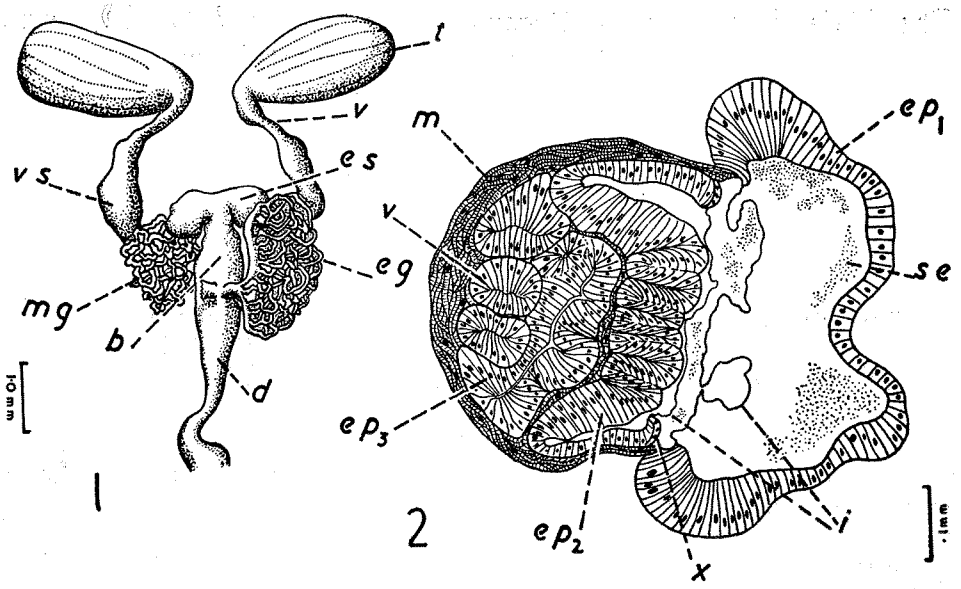
The Mature Organs

The male reproductive organs comprise paired testes, vasa deferentia, vesiculae seminales, a complex bulbus and a ductus ejaculatorius. Mesodermal and ectodermal accessory glands are also present.

The testes are elongate ovoid in form and lie across the body cavity. Each has six flattened follicles, this number being found in all the Pentatominae examined. From the base of the testis, and at right angles to it, arises the vas deferens. This passes backwards and slightly outwards until the level of the apex of the bulbus is almost reached, when it bends at right angles and passes to this organ. Its middle parts are somewhat swollen and constitute the vesicula seminalis.

The angle between the basal parts of the vas and the bulbus is occupied by a large mass of narrow, tangled tubules of the mesodermal accessory gland or mesadene (Fig. 1, mg). Each tubule has an epithelium of large, more or less cuboidal cells with convex, somewhat indefinite, distal borders (Fig. 7). With Bouin's fixative, the cytoplasm is coarsely granular and strongly eosinophilic. The nuclei are large, and evenly and densely granular. The lumen of the tubule is entirely filled with an eosin-stained secretion of both large and small granules.

The bulbus ejaculatorius is very long, fusiform, and is of very complex construction. It is partly covered by an epithelium here termed the investing epithelium

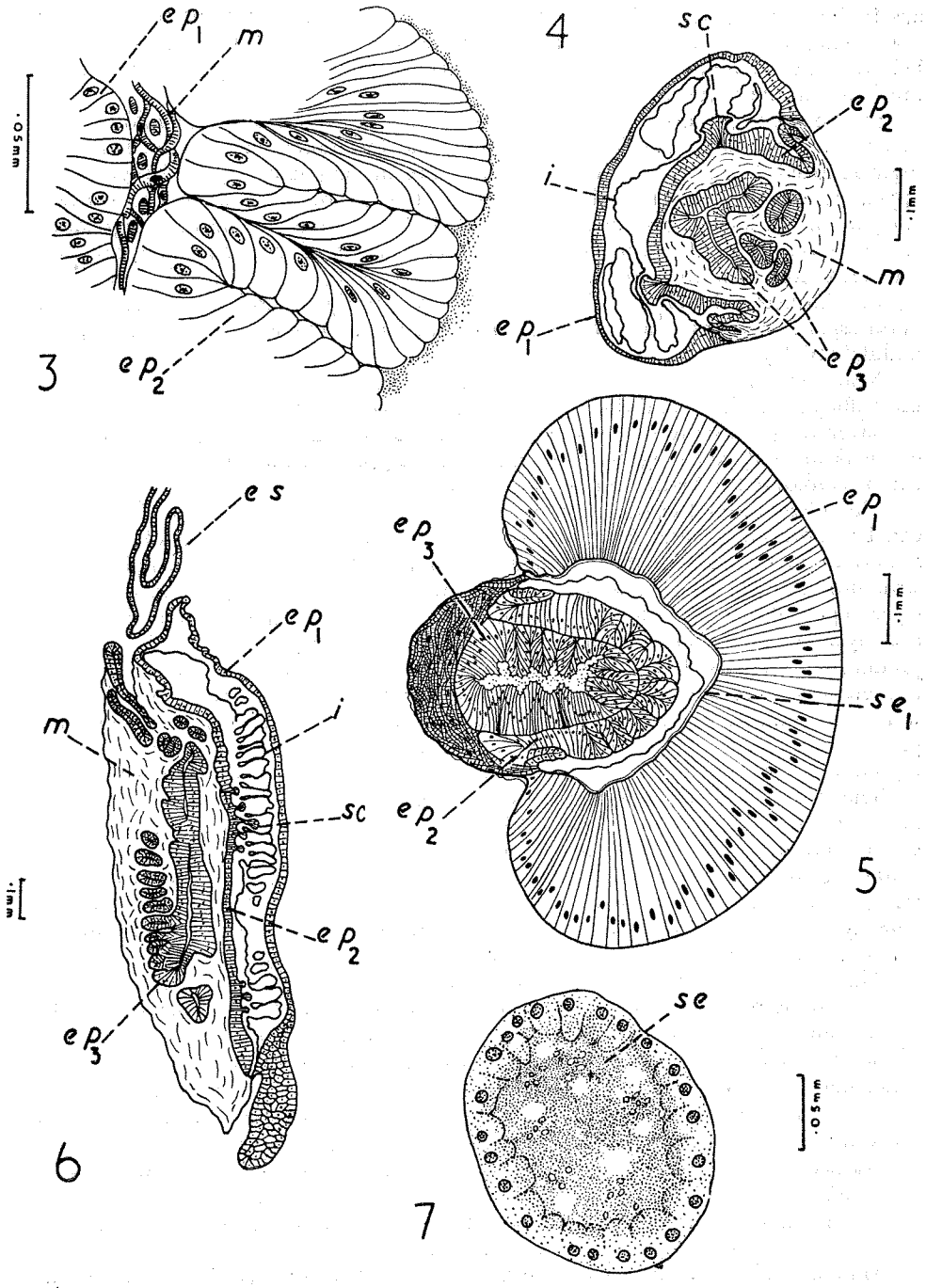


TEXT-FIG 1.—Fig. 1—Male reproductive organs. Left ectadenes omitted to show mesadenes beneath. Fig. 2—T.S. anterior part of bulbus ejaculatorius.

(ep_1) which is continuous with that of the ductus ejaculatorius. This secretes a chitinous intima which usually becomes separated from the cell layer and lies loosely outside the more central parts of the organ. Within this and separated by a space from the investing layer, are two more or less concentric epithelial tubes forming a central core to the organ. The outer one, termed the middle epithelial layer (ep_2), consists of secretory cells which discharge into the space within the investing layer. The inner layer, the lining epithelium (ep_3), is also composed of secretory cells, but their products enter the central lumen of the bulbus. Into the dorsal wall of the bulbus at its widest part open the paired ectodermal glands or ectadenes (Fig. 1, eg).

Anteriorly, dorsally and posteriorly the organ is covered by the investing epithelium (Fig. 2, ep_1). At the apex of the bulbus this is produced to form a dilated bi-lobed ectodermal sac (Fig. 1, es). Here the cells are flattened, have large nuclei and are definitely secretory in character. A layer of the secretion remains closely applied to the free borders of the cells. This layer is somewhat refractile and, considering the ectodermal nature of the epithelium, might be considered to be the chitinous cuticle, although its borders do not have the sharp character typical of such an intima. Within this layer are masses of coagulated secretion. Dissections show that in the unfixed condition this sac is filled with a clear, watery secretion.

The ventral and lateral aspects of the bulbus are covered by a thick coating of muscle fibres (Fig. 2, m) which are extended as a thin layer between the middle epithelium (ep_2) and the lining epithelium (ep_3), thus completely surrounding the latter. The lining epithelium forms a tube of extremely long, narrow cells which almost completely obscure the lumen. These are glandular, especially in the more posterior regions, and pass masses of secretion into the lumen. Anteriorly, the combined vasa and mesadene ducts enter this central canal (Fig. 2, v). The middle epithelium (Fig. 2, ep_2) is much folded and except for its lateral extremities, is composed of tall cells (Fig. 3). Laterally, it almost meets the investing cell layer (ep_1), but is separated from it by the dorsal ends of the muscle layer. At each end it bends inwards slightly, and the most dorsal cells (Fig. 2, x) which are very reduced,



TEXT-FIG. 2.—Fig. 3—Detail of Fig. 2 to show folds of middle epithelium. Fig. 4—T.S. bulbus ejaculatorius to show active groups of secretory cells of middle epithelium. Fig. 5—T.S. bulbus posterior to that shown in Fig. 2. Fig. 6—Vertical L.S. bulbus somewhat laterad of mid-line. Fig. 7—T.S. mesadene tubule.

are folded back on to it. The intima is a thin, extremely contorted structure best investigated from longitudinal sections (Figs. 6, 11). This will be further discussed below. Both the middle and investing epithelia are glandular, and masses of secretion are found in the dorsal space (Fig. 2, se).

In the more posterior regions the investing epithelium and the middle layer progressively surround the lining epithelium, and the muscle layer becomes increasingly reduced (Figs. 5, 8). These changes are accompanied by a change in the appearance of the cells of the investing layer (Fig. 5, ep_1). These undergo extreme elongation, the longest reaching a length of 0·25 mm. There is also an aggregation of the secretions of this cell layer on the free borders of the cell. This results in the formation of a thin, refractile border (Figs. 5, 8, 9; se_1) which has all the optical characteristics of a chitinous intima. Posteriorly the intima (i) lying in the dorsal space becomes less contorted and loses its attachment to the ends of the middle cell layer.

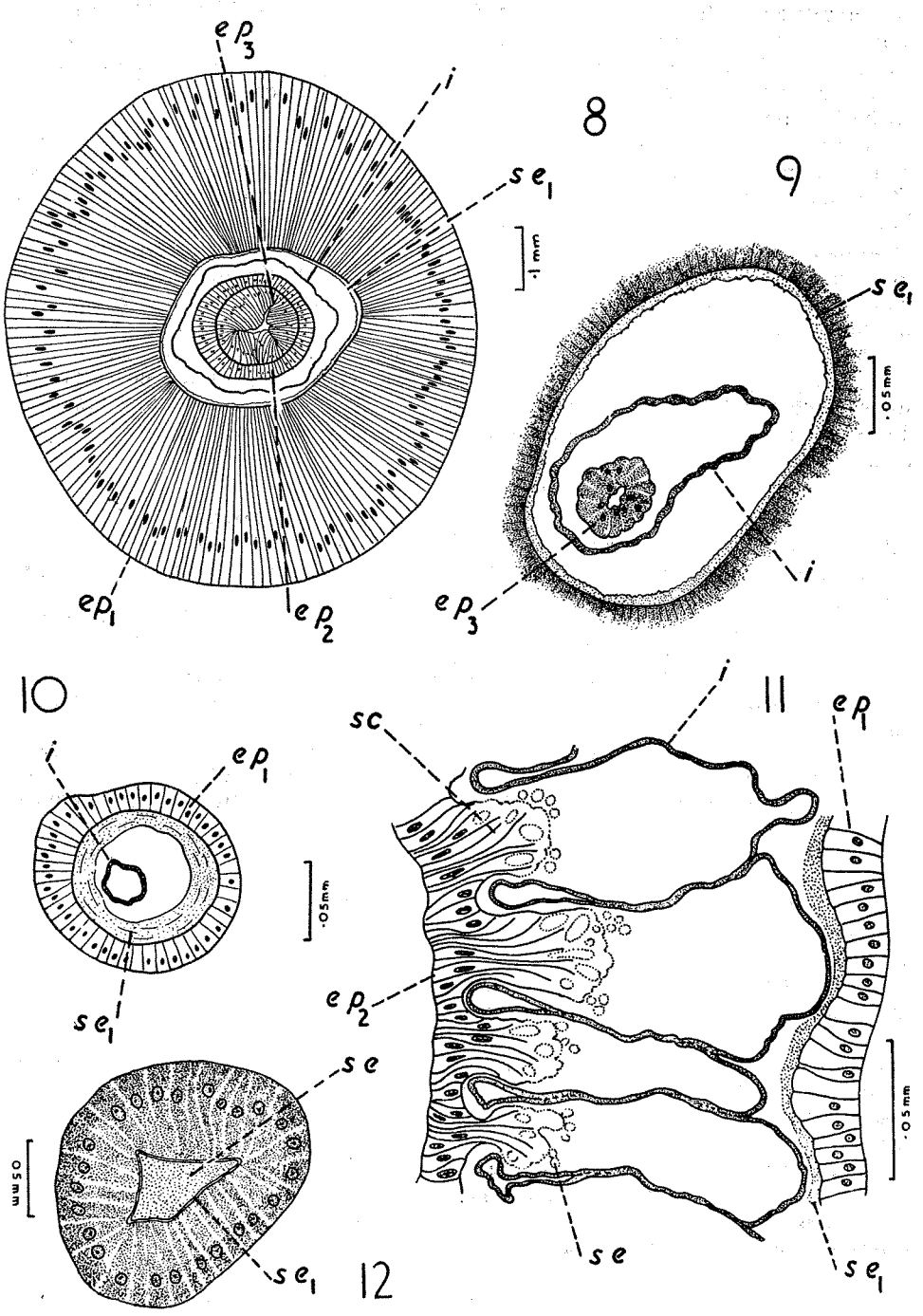
Near the base of the bulbus the investing layer surrounds the central parts of the bulbus as in Fig. 8, and the middle epithelium (ep_2) completely invests the lining cell layer (ep_3). The muscle coating is absent. At the base of the organ the central epithelia are reduced (Fig. 9, ep_3) and soon disappear. From this point the organ can be considered to be the ductus ejaculatorius.

When longitudinal sections are prepared the character of the intima of the bulbus can be appreciated. Fig. 6 represents a vertical longitudinal section of the organ somewhat to one side of the mid-line. The intima can be seen as a much-folded structure occupying the space between the investing cell layer (ep_1) and the middle layer (ep_2). Projecting into the areas between the folds of intima from the middle cell layer are mushroom-like masses of secreting cells (Fig. 11, sc). The secretion produced must pass down on the inside of the intima and thus enter the intromittent organ via the chitinous tube of the ductus. These masses of secreting cells were not found as a constant feature in all specimens examined by sectioning, their presence or absence probably depending on the phase of secretory activity of the reproductive organs. Fig. 4 illustrates their appearance in transverse section (sc).

The ductus ejaculatorius consists of an epithelium of cells which become progressively cuboidal posteriorly (Fig. 10), and a central chitinous tube carrying the sperm and secretions of the mesadenes, the lining epithelium and the middle epithelium of the bulbus. The space outside the chitinous tube must provide access for the secretions of the investing epithelium and of the ectadenes (see below). The layer of dense secretion which covers the free borders of the epithelium thickens considerably in the ductus and has the appearance of a heavy chitinous lining.

The ectadenes enter the investing layer of the bulbus in the mid-dorsal line anterior to the region where the investing epithelium cells become considerably taller. Each gland comprises a dense mass of tubules which are slightly thinner than those of the mesadenes. These pour their secretions into anterior or posterior longitudinal collecting canals which join to form a very short, wide duct entering the dorsal space of the bulbus. The epithelium of the glands is continuous with that of the investing epithelium of the bulbus, developmental studies showing that the tubules are formed as a series of lateral diverticula of this epithelium. Each tubule has an epithelium of tall, narrow cells (Fig. 12) with strongly cyanophilic cytoplasm. A very thin, slightly refractile layer of secretion covers the distal borders of the cells (Fig. 12, se_1). The small lumen is packed with a finely granular secretion.

Malouf (1933) has described the male reproductive organs of *Nezara viridula*. He has figured a transverse section (Plate VII, Fig. 10) of the organ which has been taken from approximately the same level as that illustrated in Fig. 2 of this present work. He claims that the duct of the bulbus is filled with spermatozoa with their heads imbedded in a matrix and directed outwards. Surrounding them is a layer of longitudinal muscle fibres, and outside these, on the dorsal side, is a layer



TEXT-FIG. 3.—Fig. 8—T.S. bulbus posterior to that shown in Fig. 5. Fig. 9—T.S. extreme posterior end of bulbus. Bases only of the cells of ep_1 shown. FIG. 10—T.S. ductus ejaculatorius. Fig. 11—Detail of Fig. 6, showing relationships between secretory cells and intima. Fig. 12—T.S. ectadene tubule.

of spermatozoa with their heads directed towards the centre. The whole structure is invested by a nucleated peritoneal membrane which on the dorsal surface is composed of columnar cells, while ventrally it forms a syncytium. Dorsally, between the columnar peritoneal layer and the dorsal layer of spermatozoa, is a space containing tracheal branches.

Examination of Malouf's figure has convinced the present writer that the former is in error in his interpretation of his sections of the bulbus. The tall, narrow cells of the lining epithelium have been misinterpreted as regularly arranged spermatozoa. Similarly, the tall cells of the regularly folded middle epithelium were mistaken by Malouf for sperm with their heads directed inwards. Malouf's columnar peritoneal layer on the dorsal side is the layer of columnar cells of the investing epithelium, while his "tracheal branches" are clearly the folds of the intima lying freely in the dorsal space.

Qadri (1949) has given a very brief description of the male reproductive organs of *Nezara viridula* and *Bagrada picta* (Fabricius). He has not described or figured the ectadenes nor has he realised the complex nature of the bulbus ejaculatorius. He describes this region as the highly swollen proximal end of the common ejaculatory duct.

Development of the Bulbus

The development of the bulbus ejaculatorius of the Heteroptera has yet to be fully investigated. Preliminary studies of its development in *Nezara* have been made by sectioning third, fourth and fifth instar nymphs, mainly to determine whether mesodermal epithelial elements enter into the composition of the bulbus.

In the third instar the ectodermal ductus ejaculatorius is a simple tube which ends blindly within. Closely applied to its anterior end, but not opening into it is the pair of vasa deferentia. There is no indication of the developing bulbus.

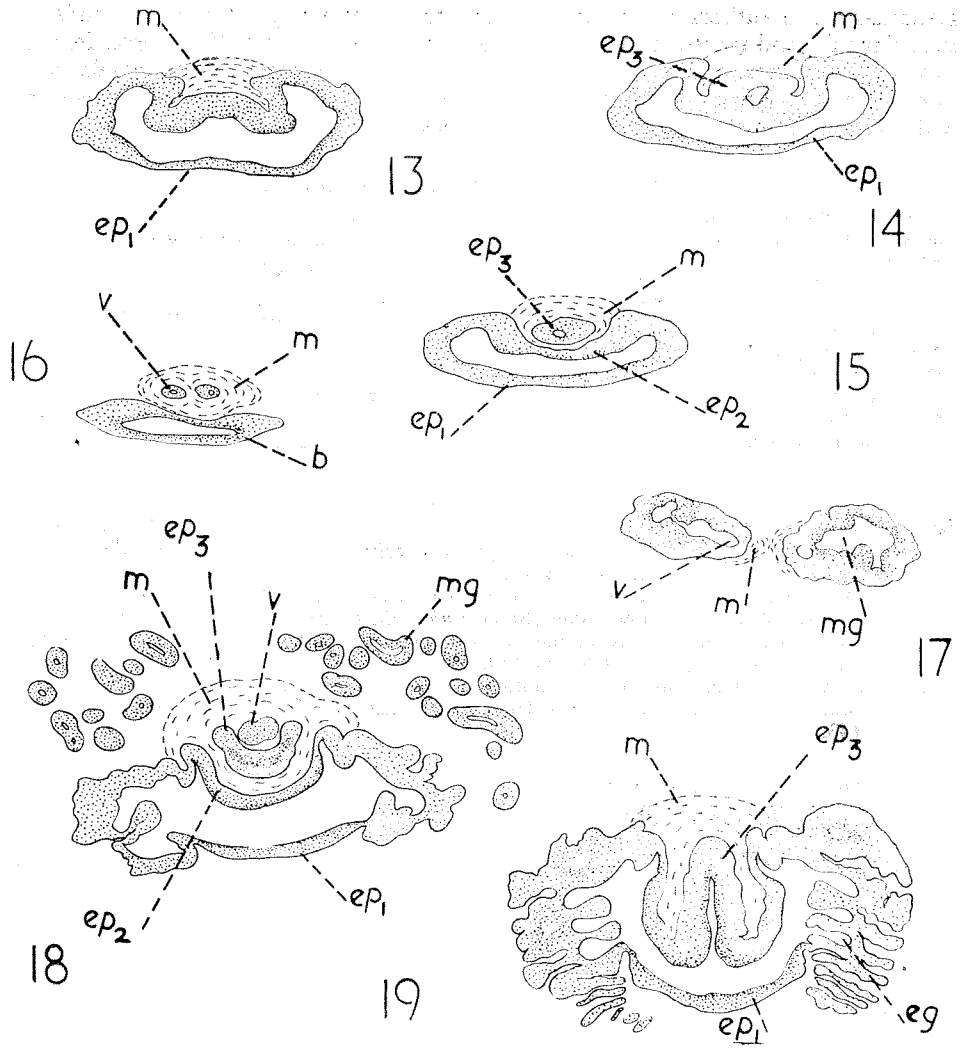
In the fourth instar early stages in the development of the bulbus are apparent. There is a longitudinal invagination of the ventral side of the more anterior parts of the ductus to form a tube having a U-shaped section (Fig. 13). The region between the arms of the U contains muscle fibres. The dorsal wall is the rudimentary investing epithelium of the mature bulbus and the ventral invaginated wall produces the middle epithelial layer. Anteriorly an evagination of the middle of the ventral wall produces a small tube surrounded by the muscle fibres (Figs. 14 and 15). This is the lining epithelium of the adult bulbus. Applied to it are the mesodermal vasa deferentia, but these do not open into it at this stage. In front of the rudimentary bulbus the developing mesodermal accessory glands are present as outgrowths of the epithelia of the vasa (Fig. 17).

In the fifth stage nymph there is an increase in the size of the bulbus, but the three layers are still clearly continuous as in the preceding instar. Although the vasa pass through the muscle layer to become closely apposed to the lining epithelium, they do not open into the bulbus (Fig. 18). At this stage the developing ectodermal glands are present as a long series of lateral outgrowths of the dorsal wall of the organ (Fig. 19), although in the adult the glands enter by a single duct on each side.

Thus, it can be concluded that all the epithelia of the bulbus ejaculatorius and the ectadene accessory glands are produced by the elaboration of the wall of the ductus and therefore are of ectodermal origin. The only mesodermal elements of the bulbus are the vasa deferentia and the muscular sheath.

Discussion

Like other land bugs, *Nezara* has a definite bulbus ejaculatorius. In the water bugs and the water-surface bugs (Gerroidea) such a bulbus is not present and the vasa open into the simple, sometimes dilated, end of the ductus ejaculatorius. It is intended to deal with these points in detail in another paper.



TEXT-FIG. 4.—Figs. 13–16—Fourth instar. Transverse sections of developing bulbus. Fig. 13 is most posterior; 16, most anterior. Fig. 17—Fourth instar, T.S. vasa and mesadenes. Fig. 18—Fifth instar, T.S. anterior end of bulbus. Fig. 19—Fifth instar, T.S. posterior end of bulbus.

The male reproductive organs of most Pentatomidae are complex and closely resemble those of *Nezara*. However, in the Acanthosominae, usually regarded as a sub-family of the Pentatomidae, the ectadenes and ectodermal sacs are missing. This lends support to the conclusion reached by Leston (1953) that the Acanthosominae should be removed from the Pentatomidae and accorded family status.

These studies have not thrown any light on the function of the massive ectadenes whose secretions, it will be recalled, pass along the ductus on the outside of the tubular chitinous intima. The sperm and mesadene secretions on the other hand, pass along on the inside of the tube to the intromittent organ. However, since the completion of this work a paper of some interest has appeared describing functional studies on the reproductive organs in the Lygaeidae (Bonhag & Wick, 1953). In this family there are no ectadenes, but the investing epithelium of the bulbus is

glandular. The authors claim that its secretion passes along the ductus outside the cuticular tube and is responsible for the erection of the aedeagus. If, as seems feasible, this is the function of the ectadene secretions in the Pentatomidae it would be of interest to attempt to correlate the structure and mode of action of the aedeagus with the degree of development of the ectadenes.

Reference Lettering to the Figures

b—bulbus ejaculatorius.
d—ductus ejaculatorius.
eg—ectadene accessory gland.
ep_1—outer epithelium of the bulbus. It is continuous with the epithelium of the ductus.
ep_2—middle epithelial layer.
ep_3—inner layer, lining the lumen of the bulbus.
es—sac of ectodermal origin.
i—cuticular intima.
m—muscle fibres.
mg—mesadene accessory gland.
sc—secretory cells.
se—secretion.
se_1—dense compacted layer of secretion.
t—testis.
v—vas deferens.
vs—vesicula seminalis.
x—reduced lateral cells of middle epithelium.

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